Open Literature Review

1. Chemical Names: Clothianidin, Dinotefuran

2. PC Codes: 044309, 044312

3. CAS Nos. 210880-92-5, 165252-70-0

4. MRID: None

5. ECOTOX Record Number and Citation:
Yamada, T., K. Yamada, and N. Wada. 2012. Influence of dinotefuran and clothianidin on a bee colony. *Japanese Journal of Clinical Ecology*. V21(1): 10—23

6. Purpose of Review: Thiamethoxam and clothianidin re-evaluation for pollinators

7. Date of Review: 11/27/14

8. Description of Use: Invalid. The study is not scientifically sound and should not be used in risk assessment.

9. Summary of Study Findings:

Executive Summary
The study authors examined the effect of three concentrations of two neonicotinoid treatments (1—10 ppm dinotefuran, 0.4—4 ppm clothianidin) provided in food sources (sugar syrup and pollen paste) for up to four months on honey bee (*Apis mellifera*) colonies. The highest treatments were administered in food for the first 12 days, while the middle and lower treatments were continually administered in food for the duration of the experiment. Colony endpoints measured included the change in number of adult bees, brood and total pesticide consumption. The study authors also performed NMR spectral analysis of the two compounds to examine their stability to temperature and UV-light. For the purpose of this data evaluation record, the reviewer evaluated the effects data presented in this study, but not the accompanying stability data. The study authors reported that all colonies exposed to neonicotinoid treatments at the rates specified had increased mortality and decreased brood production compared to controls and eventually became extinct by 68 days. Colonies in the highest treatments that were only fed contaminated food sources during the first twelve days persisted longer than the colonies that were continually fed lower and middle dietary concentrations. The study authors propose that the colonies' reactions to these neonicotinoid concentrations may be similar to that observed in colonies identified as suffering from CCD (Colony Collapse Disorder)

Methods

Pesticide formulations used were STARKLE MATE® (containing 10% dinotefuran) and DANTOTSU® (16% clothianidin). Neither of these formulations is registered for use in the United States or Canada. The study authors described using these formulations to create solutions with a dilution factor recommended for exterminating stinkbugs, using a 1,000-fold and 4,000-fold dilution factor, to obtain a commercial concentration in sugar syrup of 100 ppm and 40 ppm, respectively for dinotefuran and clothianidin. These solutions were then further diluted 10-, 50-, and 100-folds to obtain the final solutions used in each trial run. Sugar syrup solutions were 50% water. Pollen paste was prepared using 2 parts pollen (one part pure pollen and one part pollen substitute, "Feed-Bee®" with one part sugar syrup (also containing control or treatment group concentrations of each pesticide).

The study authors started experiments with one colony of 10,000 bees per control or treatment group. There were two control groups, three dinotefuran treatment groups (1 ppm, 2 ppm, and 10 ppm nominal) and three clothianidin groups (0.4 ppm, 0.8 ppm and 4 ppm) as described in **Table 1** (reproduced from Yamada et al, 2012). Sugar syrup in a feeder and pollen as a paste placed directly on combs were fed to each colony. Colonies were fed every 5-10 days (less frequently after September 24) with control or treatment solutions, however in the high (10 ppm dinotefuran and 4 ppm clothianidin) trial runs, uncontaminated sugar syrup and pollen paste were fed to the colonies starting with the third assessment date (July 30).

Commented [WM1]: Study authors describe the frequency of feeding and replacing old feed as "every time". I'm not sure what this means—maybe every observation period, which would mean every 5-10 days until September 24 with increasing time to replacement from then until experiment end on November 21.

Table 1. Outline of foods (sugar syrup, pollen paste) on each experimental run

Run	Formulation	Dilution[1] of Product	Dilution of Ref. Sol.[2]	Concentration[3]	Notation[4]	Note[5]
RUN01	No pesticide			0 ppm	B-1 (Blank run)	(control)
RUN02	Starckle™ (dinotefuran 10%)	10,000-fold dilution	10	10 ppm	$S^{10}{}_{1000}$	S-high
RUN03	Starckle™ (dinotefuran 10%)	50,000-fold dilution	50	2 ppm	$S^{50}{}_{50000}$	S-middle
RUN04	Starckle™ (dinotefuran 10%)	100,000-fold dilution	100	1 ppm	$S^{100}{}_{100000}$	S-low
RUN05	Dantotsu™ (clotianidin 16%)	40,000-fold dilution	10	4 ppm	$D^{10}{}_{4000}$	D-high
RUN06	Dantotsu™ (clotianidin 16%)	200,000-fold dilution	50	0.8 ppm	$D^{50}{}_{200000}$	D-middle
RUN07	Dantotsu™ (clotianidin 16%)	400,000-fold dilution	100	0.4 ppm	$D^{100}{}_{400000}$	D-lo
RUN08	No pesticide			0 ppm	B-2 Blank	

[1] Dilution of Product means that a commercial pesticide is diluted with sugar syrup up to a given dilution factor.
[2] Dilution of Ref. Sol. Represents a dilution factor diluting the reference solution which is recommended as a concentration for the extermination of stinkbugs, where the reference solution of Starcklemate™ and Dantotosu™ have a 1,000-fold dilution of a commercial product (dinotefuran of 100 ppm in solution) and a 4,000-fold dilution of a separate product (clothianidin of 40 ppm in solution), respectively.
[3] Concentration represents the concentration of pesticide nominally administered to each run.
[4] B-1 and B-2 represent blank runs (controls). X and Y in $S^{x}{}_{y}$ and $D^{x}{}_{y}$ represent the X-fold dilution of the reference solution and the Y-fold dilution of the commercial product, respectively, and the S and D represent Starcklemate™ and Dantotsu™, respectively.
[5] High conc. (concentration), middle conc., and low conc. Means a 10-fold dilution of the reference solution, a 50-fold one and a 100-fold one, respectively, in Yamada *et al.*, 2014.

Colonies were placed in an apiary (entrances all facing east) where bees were able to freely forage on flowers in the field. Each hive started with six frames and one feeder of sugar syrup. Additional frames with blank foundation were added to control hives when necessary. The study authors did not report the date that hives were originally placed in the apiary, but the initial colony assessment was reported to be July 18, 2010 and assessments continued until November 21, 2010. Colony assessments were conducted in the early morning before bees were out foraging, generally at weekly intervals, but sometimes at biweekly intervals.

Colony assessments were conducted using digital photography. Pictures were taken of both sides of each comb (frame), the feeder, insides and outside the hive at weekly intervals. Pictures of the vicinity around the hive entrance were taken with a half-hour intervals timer to monitor honey bee activities. The number of adult bees on a frame were directly counted on a photo when it was less than several hundred. In cases where more adults were present, the study authors compared the taken photos with reference photos that had been previously directly counted. The total number of adult bees in each hive was considered the sum of all the bees on both sides of each frame. The amount of brood was evaluated from photos by considering the ratio of area containing brood to the entire surface on each side of a frame and then summed across all frames in the hive. Consumption of sugar syrup and pollen paste and the number of dead adults were estimated from both photos and visual measurements.

Since colonies started with different populations, the study authors developed a metric for the change in the number of adult bees in an experimental run compared to the average change in bee numbers in the control runs, to facilitate comparison across trial runs. This was defined by the following equation:

Normalized number of adult bees = $(n_{ij}/n_{i0})/(n_{Bj}/n_{B0})$

n_{ij} = number of adult bees in RUN *i* after the elapse of *j* days
n_{i0} = initial number of adult bees in RUN *i* at the start of experiment
n_{Bj} = mean number of adult bees in blank runs (controls) after the elapse of *j* days
n_{B0} = mean initial number of adult bees in blank runs at the start of the experiment

Statistics

No statistical analysis was performed on any of the data by the study authors. This is likely a result of using only one hive/treatment which precludes the ability to quantitatively analyze the data.

Results

Control colonies gained approximately 18% adult bees between the first and second colony assessments (5 days after experimental start) and approximately 3% at 68 days after experimental start (**Table 2** and **Figure 1**, reproduced from Yamada *et al*,. 2012). In comparison, by the second colony assessment, dinotefuran treatments lost approximately 24%, 59% and 53% of initial adult bee numbers in the 1 ppm, 2 ppm and 10 ppm treatments, respectively and had lost approximately 87%, 100% and 96% of initial adult bee numbers in the 1 ppm (S-low), 2 ppm (S-middle) and 10 ppm (S-high) treatments by Day 68. Clothianidin treatments lost approximately 11%, 33% and 60% of initial bee numbers in the 0.4 (D-low), 0.8 (D-middle) and 4 (D-high) ppm treatments, respectively by the second colony assessment. By day 68, clothianidin treatments had lost 99%, 100% and 93% of initial bee numbers in the 0.4, 0.8 and 4 ppm treatments, respectively by Day 68.

Commented [WM2]: Study authors also describe that after July 18, there are few flowers in bloom.

Commented [WM3]: Though for some reason, assessments on controls stopped after September 24.

Commented [WM4]: Unclear to me whether this was done during the weekly intervals only, or if it was all the time.

Commented [WM5]: Study authors stopped control colony assessments after this time

In the high treatments, the study authors reported that shortly after the initial contaminated food administration (these colonies were only fed treated foods for 12 days), a “great number of dead bees” were found around the hive for 12 days while after this period only “a few” to “some” dead bees were found around the colony. These colonies became extinct in 15 weeks for the 10 ppm dinotefuran treatment and 18 weeks later in the 4 ppm clothianidin treatment. Queens persisted in these treatments until all adult bees had died. In the middle treatments (2 ppm dinotefuran and 0.8 ppm clothianidin) the queens persisted until bee numbers were low (0.6—1.4% of initial adult bee numbers). In these treatments, the study authors reported that a “number” of dead bees occurred in the early period after contaminated food administration, but “almost never” occurred afterwards. In the low treatments (1 ppm dinotefuran and 0.4 ppm clothianidin), queens persisted until all bees had died in the low dinotefuran treatment and until 14% of the initial bees were present in the low clothianidin treatment.

Table 2 Change in number of total adult bees with elapsed days for each run

Date in 2010	Elapsed days	RUN 1 *control*	RUN 2 *S-high* (10 ppm)	RUN 3 *S-middle* (2 ppm)	RUN 4 *S-low* (1 ppm)	RUN 5 *D-high* (4 ppm)	RUN 6 *D-middle* (0.8 ppm)	RUN 7 *D-low* (0.4 ppm)	RUN 8 *control*	Average of Blanks
(Pesticide)		Blank 1	Starcklemate™	Starcklemate™	Starcklemate™	Dantotsu™	Dantotsu™	Dantotsu™	Blank 2	Blank 1 & 2
(Dilution[1)])		No pesticide	10,000-fold[1)]	50,000-fold[1)]	100,000-fold[1)]	40,000-fold[1)]	200,000-fold[1)]	400,000-fold[1)]	No pesticide	No pesticide
July 18	0	8950	11700	12720	10400	12880	11600	13400	10560	9755
July 23	5	11700	5450	5240	7900	5100	7800	11900	11400	11550
July 30	12	11850	(3900)	7250	8750	(1770)	8900	12100	11800	11825
August 8	21	11100	(2550)	1235	9500	(1775)	4060	10100	12400	11750
August 13	26	11400	(1450)	940	8500	(1530)	[70]	9900	11800	11600
August 21	34	8900	(861)	325	4750	(640)	[275]	6300	10700	9800
August 26	39	9800	(980	200	5150	(890)	[36]	4340	9400	9600
September 5	49	9650	(760)	[178]	4590	(830)	[0]	[1840]	6370	8010
September 11	55	10600	(666)	[110]	3550	(810)		[1180]	7450	9025
September 17	61	11150	(264)	[0]	3740	(730)		[975]	6150	8650
September 24	68	12300[2)]	(470)		1395	(895)		[150]	7680[2)]	9990
October 10	84	12300[2)]	(415)		0	(740)		[0]	7680[2)]	9990
October 30	104	12300[2)]	(0)			(285)			7680[2)]	9990
November 21	126	12300[2)]				[(0)]			7680[2)]	9990

[1)] This shows a dilution factor of a commercial product. [2)] The numbers of adult bees on the elapsed of 68 days in RUN 1 & 8 were substituted for that after that.
(Note) Parentheses ( ) show a state that foods (sugar syrup, pollen paste) without a pesticide were fed into a colony after the elapse of 12 days instead of foods with a pesticide. Brackets [ ] show a state that a queen had been lost. The average between RUN 1 & 8 was used as the number in blank run in calculation of normalized number. Starcklemate™ contains a dinotefuran content of 10% and Dantotsu™ contains a clothianidin content of 16%. Less than ten heads are expressed as zero.

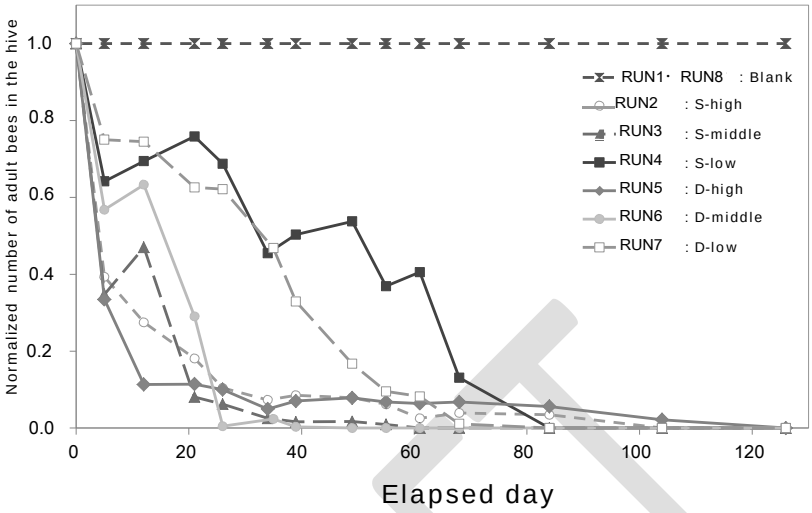

Figure 1. Normalized number of adult bees in the hive with the elapsed days

Control colonies had a decrease of approximately 21% in total brood area between the first and second colony assessment dates, with progressively decreasing brood areas until Day 49, when the decrease in brood area was approximately 71%, compared to starting brood area followed by rebounding brood numbers by Day 68 with a final brood area approximately 33% smaller than the starting brood size (**Table 3** and **Figure 2**, reproduced from Yamada *et al*., 2012). In comparison, by the second colony assessment date, dinotefuran exposed colonies lost approximately -4%, 45% and 37% of initial brood area in the S-low, S-middle and S-high treatments, respectively and by Day 68 had lost 100%, 100% and >99% of initial brood area in the S-low, S-middle and S-high treatments. Colonies in the clothianidin-exposed treatments lost approximately -5%, 7%, and 66% initial brood area, respectively in the D-low, D-middle and D-high treatments by the second assessment date and lost approximately 100%, 100% and >99% of initial brood areas, respectively in the D-low, D-middle and D-high treatments by Day 68. The study authors reported that in some colonies there was a "peak" in brood approximately five weeks after application, which they suggested was caused by stimulation in egg-laying of a queen due to the sharp decrease in the number of brood. Generally, the reviewer found this "peak" in production to be relatively small compared to initial brood areas. The study authors observed that in the high concentration treatments, where uncontaminated syrup and nectar were fed after the initial 12 days of exposure, the queens egg-laying capacity never recovered and remained impaired through the remainder of the experiment. The study authors suggest that the diminishment of brood observed in this article, caused by the pesticide application results in a state of CCD (Colony Collapse Disorder) to the colony.

Table 3. Change in number of total brood area in a hive with elapsed days for each run

Date in 2010	Elapsed Days	RUN 1 *control*	RUN 2 *S-high*	RUN 3 *S-middle*	RUN 4 *S-low*	RUN 5 *D-high*	RUN 6 *D-middle*	RUN 7 *D-low*	RUN 8 *Control*	Average of Blanks
(Pesticide)		Blank 1	Starcklemate™	Starcklemate™	Starcklemate™	Dantotsu™	Dantotsu™	Dantotsu™	Blank 2	Blank 1 & 2
(Dilution[1)])		No pesticide	10,000-fold[1]	50,000-fold[1]	100,000-fold[1]	40,000-fold[1]	200,000-fold[1]	400,000-fold[1]	No pesticide	
July 18	0	5.3	7.05	7.2	3.9	7.6	1.5	2	6.96	6.13
July 23	5	5.25	4.45	3.95	4.05	2.6	1.4	2.1	4.45	4.85
July 30	12	3.7	(0.8)	1.4	1.35	(0.25)	0.05	0.05	3.5	3.6
August 8	21	2.5	(0.4)	0	0.05	(0.2)	0.15	0.3	3.45	2.975
August 13	26	2	(0.4)	0	0.1	(0.05)	[0.2]	0.35	2.95	2.475
August 21	34	2.55	(0.4)	0.07	0.6	(0.3)	[0.006]	0.8	2.8	2.675
August 26	39	2.5	(0.15)	0.1	0.25	(0.4)	[0]	0.235	2.05	2.275
September 5	49	1.4	(0.05)	[0.039]	0.036	(0.05)	[0]	[0.049]	2.1	1.75
September 11	55	1.5	(0.065)	[0.065]	0.008	(0.098)		[0]	2.6	2.05
September 17	61	1.9	(0.042)	[0.042]	0.005	(0.099)		[0]	3	2.45
September 24	68	3.85[2)]	(0.016)		0	(0.045)		[0]	4.4	4.125[2)]
October 10	84	3.85[2)]	(0.06)		0	(0.141)		[0]	4.4	4.125[2)]
October 30	104	3.85[2)]	(0)			(0.026)			4.4	4.125[2)]

[1]Dilution shows a dilution factor of a commercial product.

[2] The mean numbers of brood on the elapsed of 68 days in RUN 1 & 8 were substituted for that after September 24.

(Note) Parentheses ( ) show a state that foods (sugar syrup, pollen paste) without a pesticide were fed into a colony after the elapse of 12 days instead of foods with a pesticide.

Brackets [ ] show a state that a queen had been lost.

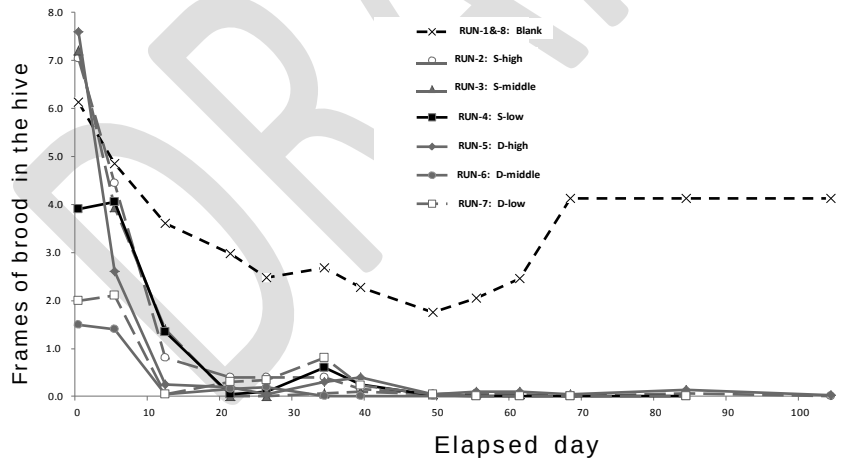

Figure 2. Change in the number of brood expressed by the total number (sides) of frames occupied by brood in the hive by elapsed day.

The study authors determined total pesticide intake per treatment (**Table 4** and **Figure 3**, reproduced from Yamada *et al*., 2012) and calculated, using an assumption of 500 honey bees/colony develop from larvae into adult bees while fed stored food products contaminated with dinotefuran or clothianidin, a total intake of 0.1072 µg/bee, 0.2434 µg/bee and 0.1903 µg/bee for S-high, S-middle and S-low, respectively and a total intake of 0.0360 µg/bee, 0.1150 µg/bee and 0.0706 µg/bee for D-

Commented [MWagman6]: I don't know where this assumption comes from.

high, D-middle and D-low treatments, respectively. The study authors conclude that since there was little difference between total dinotefuran intakes between the dinotefuran middle and low treatments and little difference between total clothianidin intakes between the clothianidin middle and low treatments, that these pesticides may be little metabolized and accumulate in the body tissues of bees leading to eventual colony collapse.

Table 4. Total intake of pesticide for each run calculated from the intake of foods

Fiducial concentration	Total intake of pesticide	RUN 1	RUN 2	RUN 3	RUN 4	RUN 5	RUN 6	RUN 7	RUN 8
		Control	S-high	S-middle	S-low	D-high	D-middle	D-low	Control
Reference solution[a] [g]	from sugar syrup [g]	0	63.3	99.8	95	63.2	98.8	93.2	0
	from pollen paste [g]	0	5	5.4	4.7	5.2	5.2	4.8	0
	from both foods [g]	0	68.3	105.2	99.7	63.4	104	98	0
Commercial product[b] [mg]	from sugar syrup [mg]	0	63.3	99.8	95	15.8	24.7	23.3	0
	from pollen paste [mg]	0	5	5.4	4.7	1.3	1.3	1.2	0
	from both foods [mg]	0	68.3	105.2	99.7	17.1	26	24.5	0
Active ingredient[c] [mg]	from sugar syrup [mg]	0	6.33	9.98	9.5	2.53	3.95	3.72	0
	from pollen paste [mg]	0	0.5	0.54	0.47	0.2	0.2	0.19	0
	from both foods [mg]	0	6.83	10.52	9.97	2.73	4.15	3.91	0
		no pesiticide	dinotefuran			clotianidin			no pesticide

a) Total intake of pesticide solution converted into the reference solution with a concentration to exterminate stinkbugs
b) Total intake of pesticide solution with the concentration which is converted into the concentration of commercial product
c) Total intake of pesticide converted into the amount of an active ingredient which is dinotefuran for RUN-2, -3 and -4 or clotianidin for RUN-5, -6 and -7
(Note) The total intake of pesticide which was converted into the pesticide solution with a concentration of a commercial product (Starcklemate™, Dantotsu™) from the comsumption of sugar syrup or pollen paste.
Where Starcklemate™ contains a dinotefuran content of 10% and Dantotsu™ contains a clotianidin content of 16%.

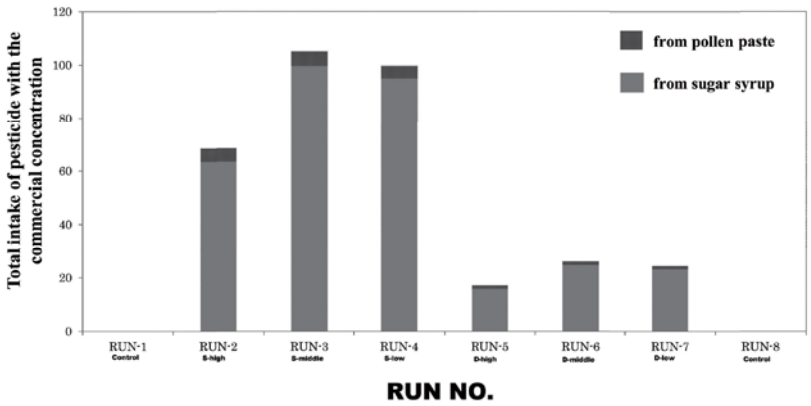

Figure 3. Total intake of pesticide with a converted concentration into that of commercial product for each run

Data Quality Evaluation

Existing guidelines are not available for honey bee colony control performance over the course of a season, however, mean adult bee numbers in the control groups seem relatively reasonable for a honey bee hive starting with 10,000 adult bees. The study authors observed a steady decrease in brood production in the controls until an uptick during the last control colony assessment on September 24 (Day 68). Typically, brood production peaks in July (as it appears to here, though brood production prior to mid-July was not reported) but an uptick in brood production would not be expected in fall, though climatic conditions and the general location in Japan where the apiary was located were not reported and may have resulted in different population dynamics than are typically observed in North America. The study authors observed and reported treatment colony data until the last treatment colony (the high clothianidin treatment) had died by Day 126, but after Day 68, control colony performance was not reported (the study authors used the Day 68 control colony numbers for all parameters on all succeeding colony assessment dates).

There were only two replicates for controls and no replication in any of the treatment groups, therefore there is considerable uncertainty into the quality of the data. As there was no replication in the study, no analytical statistics could be performed on the experimental data. In their observations of mortality around the hive entrance, the study authors used qualitative language only (*i.e.* "great number", '"a few" to "some'", and a "number") that is difficult to objectively compare.

The use of digital photography to count up to several hundred bees and to use previously counted reference photos where counts were higher is appropriate for this type of study.

10. Peer Review

Primary Reviewer Comments

Rationale for Use:

Given the lack of replication in the study and uncertainties concerning the extremely high treatment rates in this study that may be unlikely to represent environmentally relevant exposures, this study is not considered to be relevant for risk assessment purposes. The study found that single colonies exposed to nominal dietary concentrations of 1—2 ppm dinotefuran or 0.4—0.8 ppm clothianidin in nectar and pollen sources completely failed (*i.e.* population dwindled to < 10 bees) within 49—84 days. Single colonies exposed to higher nominal dietary concentrations of 10 ppm dinotefuran or 4 ppm clothianidin in nectar and pollen sources for 12 days also failed, though they persisted for 104—126 days before complete failure. All treatments consisted of one single colony per treatment only and statistical analysis could therefore not be conducted on the data. Nominal concentrations were far in excess of any from registrant submitted or currently

available open literature studies for residues in pollen and nectar over an extended period of time (*i.e.* residues in nectar and pollen beyond those present immediately after pesticide application for foliar sprays). Effects to colonies from lower daily dietary concentrations that are more likely to represent worst case scenarios cannot be determined from this study. No residue analysis was conducted on either the supplied food sources or the stored honey bee foods, so the actual concentrations to which colonies were exposed to remains uncertain. It does not appear that the specific formulations used in this study are registered in the United States and Canada and it was unclear from the study whether analogous use patterns are registered in the United States or Canada to the use pattern described in this study.

Limitations of Study:

Major flaws of this study include a lack of replication, nominal concentrations that were far higher than any previously reported from registrant submitted or open literature studies for extended periods of time and a lack of residue analysis in the treated media. Additional uncertainties associated with this study include descriptions of mortality around hive entrances that were not quantitative, lack of data around other hive endpoints (*e.g.* honey and pollen storage), and lack of control colony observations after Day 68. Although colonies were able to freely forage, if they did not want (or wanted to supplement) the supplied sugar syrup and pollen paste, the study authors reported that few flowers were in bloom. Therefore, it is unclear whether the observed colony responses were due to toxicity inherent from the test chemical or due to colony starvation due to inadequate forage.

The study authors reported an average chemical intake per bee from each treatment, though the reviewer was unable to replicate the authors' results. The study authors report that total per capita intake was 0.1072 µg/bee, 0.2434 µg/bee and 0.1903 µg/bee for the 10 ppm, 2 ppm and 1 ppm dinotefuran treatments, respectively. Dinotefuran's reported acute contact 48-hr LD_{50} is 0.024 µg a.i./bee and acute oral 48-hr LC_{50} is 0.0076 µg a.i./bee (USEPA, 2011a). Although, the total average intake reported by the study authors was over a duration far longer than that conducted in the acute tests, the average total intake per bee is an order of magnitude higher than the acute contact LD_{50} and two orders of magnitude higher than the acute oral LC_{50}. Similarly, for clothianidin the total per capita intake was reported to be 0.0360 µg/bee, 0.1150 µg/bee and 0.0706 µg/bee for the 4 ppm, 0.8 ppm and 0.4 ppm clothianidin treatments, respectively. Clothianidin's reported acute contact 48-hr LD_{50} is 0.0439 µg a.i./bee and acute oral 48-hr LC_{50} is 0.00368 µg/bee (USEPA, 2011b). The average total intake per bee is near the acute contact LD_{50} and at least an order of magnitude higher than the acute oral LC_{50} for clothianidin. If the average chemical intake in the colonies that the study authors reported is correct , it should not be surprising that each of these colonies collapsed.

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The study authors suggest that their results indicate a plausible mechanism for Colony Collapse Disorder (CCD). CCD is typically characterized by the complete loss of adult forage bees without any signs of bee mortality leaving colonies with ample brood and food reserves, along with a small cluster of hive bees including the queen (Van Engelsdorp *et al*., 2009 and Van Engelsdorp and Meixner, 2009). This characterization of CCD symptoms is similar to the effects reported in this study, though brood reserves decreased substantially after 5 days of treatment and the study authors did not report information on food reserves. Importantly, the study authors did not measure for residues of clothianidin or dinotefuran in the hives. Due to the high concentrations used, and the general persistence of these compounds, it is likely that residue analysis conducted on stored media in the hives would have found elevated levels of these compounds. However, where residue analysis has been conducted, elevated residues of clothianidin, dinotefuran and other nitroguanidine neonicotinoids have not previously been found in appreciable amounts in colonies that have been identified as suffering from CCD (VanEngelsdorp *et al*., 2009).

Description of Use in Document:
Invalid. The study is not scientifically sound and will not be used in the risk assessment.

Secondary Reviewer Comments:
[Provide any comments from secondary reviewer. Comments should be high level (e.g., related to the conclusions of the study, major flaws in design, or how it is used in risk assessment]

Resolution:
[Provide a description of the resolution if there is a discrepancy between the primary and the secondary reviewer]

11. References:

USEPA, 2011a. Registration Review—Preliminary Problem Formulation for Ecological Risk and Environmental Fate, Endangered Species, and Drinking Water Assessments for Dinotefuran. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division. D389547. Washington, D.C. December, 13, 2011.

USEPA, 2011b. Registration Review: Problem Formulation for the Environmental Fate and Ecological Risk, Endangered Species, and Drinking Water Exposure Assessments of Clothianidin. Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Environmental Fate and Effects Division. D391491. Washington, D.C. December, 13, 2011.

vanEngelsdorp, D., J.D. Evans, C. Saegerman, C. Mullin, E. Haubruge, B.K. Nguyen, M. Frazier, J. Frazier, D. Cox-Foster, Y. Chen, R. Underwood, D.R. Tarpy, and J.S. Pettis. 2009. Colony Collapse Disorder: A Descriptive Study. PLoS ONE. 4(8): e6481.

vanEngelsdorp, D. and M. Meixner. 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology. 103: S80—S95.

Yamada, T., K. Yamada, and N. Wada. 2012. Influence of dinotefuran and clothianidin on a bee colony. *Japanese Journal of Clinical Ecology*. V21(1): 10—23

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